IN THE UNITED STATES PATENT & TRADEMARK OFFICE

Applicant:	Gravereaux et al.	Group Art Unit:	1614
Serial No.:	09/970,088	Examiner:	Not Yet Assigned
Filing Date:	October 2, 2001		
For:	Use of Lymphangiogenic Agents to Treat Lymphatic Disorders		

BOX MISSING PARTS (SEQUENCE)
ASSISTANT COMMISSIONER OF PATENTS
WASHINGTON, D.C. 20231

CERTIFICATE OF EXPRESS MAIL

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal Service with sufficient postage as EXPRESS mail No. EL 789783835 US, in an envelope addressed to Assistant Commissioner for Patents, Box Missing Parts, Washington, D.C. 20231 on June 5, 2002.

By: Elizabeth Anne Sweeny
Name: Elizabeth Anne Sweeny

Sir:

PRELIMINARY AMENDMENT

Please amend the specification of the above-referenced U.S. Utility Application as follows. Marked copies of the revisions have been provided in the attached Appendix I.

In the specification:

Please replace the paragraph at page 12, lines 19-21, with the following paragraph:

--Figure 21 is a drawing showing a partial sequence of the rabbit VEGFR-3 cDNA sequence (SEQ ID NO: 7). Also shown, for comparison, are bovine (SEQ ID NO: 8), human (SEQ ID NO: 9) and mouse (SEQ ID NO: 10) sequences.--

Please replace the paragraph at page 12, lines 23-27, with the following paragraph:

--Figure 22A is a drawing showing the amino acid sequence encoded by the rabbit nucleic acid sequence of figure 21 (SEQ ID NO: 11). Also shown for comparison are bovine (SEQ ID NO: 12), human (SEQ ID NO: 13), and mouse (SEQ ID NO: 14) sequences. Figures 22B-C show results of RT-PCT experiments. Results of those experiments are summarized in Figure 22D.--

Please replace the paragraph at page 38, lines 7-31, with the following paragraph:

--Because the rabbit VEGFR-3 DNA sequence has not been disclosed, we sequenced part of the VEGFR-3 cDNA using degenerate oligonucleotides. Degenerate oligonucleotides were designed from conserved aa sequences NVSDSLEM (SEQ ID NO: 1) and WEFPRER (SEQ ID NO: 2), located 90 aa residues upstream or 40 aa residues downstream, respectively, of the trans-membrane domain of human and mouse VEGFR-3/Flt-4(Finnerty et al 1993, Galland 1993). The deduced oligonucleotide sequence were 5'-AACGTGAG(CT)GACTC(GC)(CT)T(AGCT)GA(AG)ATG-3' (SEQ ID NO: 3) and 5'-CC(GT)YTC (CT)C(GT) GGG(AG)AA(CT)TCCCA-3' (SEQ ID NO: 4), respectively. Total RNA was extracted from kidney, ear, paraaortic lymph nodes, mesentery, and lung using TRIzol(Life Technologies, Inc., Grand Island, NY, USA) according to the standard acid-guanidium-phenol-choloroform method. Two microgram of total RNA were reverse transcribed using random hexamer and Moloney murine leukemia virus reverse transcriptase(MMLV-RT) (SuperscriptII™, GibcoBRL, Life Technologies, Inc., Grand Island, NY, USA) according to the manufacturer's instructions. Briefly, the RNA was reverse transcribed in 20µl of reaction mixture containing of 10mM of each dATP, dCTP, dGTP, and dTTP; 0.1M DTT; 200U MMLV-RT, 40U Ribonuclease inhibitor and buffer. One tenth volume of the reverse transcriptase(RT) product was subjected to polymerase chain reaction(PCR) in the presence of the above-mentioned pair of oligonucleotides and Taq DNA polymerase(GibcoBRL). PCR cycles were as follows: 94°C, 2min(once); 94°C, 15 sec; 50°C, 30sec; 72°C, 1 min(30 times); 72°C, 10 min(once). A single PCR product of approximately 470 base pairs was obtained from all the tissues The PCR product from the kidney sample was subcloned into the pBluescript vector(PCR-Script Amp Cloning Kit,

Stratagene, La Jolla, CA, USA) for sequencing and probe preparation. Sequencing was performed utilizing simultaneous bidirectional-sequencing technique using Sequwncher(GeneCodes, Ann Arbor, MI)(MWG Biotech Inc., High Point, NC, USA).--

Please replace the paragraph at page 39, lines 8-14, with the following paragraph:

--Figure 21 is explained in more detail as follows. Degenerate oligonucleotides designed from conserved amino acid sequences NVSDSLEM (SEQ ID NO: 1) and WEFPRER (SEQ ID NO: 2), located 90 amino acid residues upstream or 40 amino acids downstream of the transmembrane domain of human and mouse VEGFR-3 were obtained. Reverse transciption and PCR were conducted. The resulting RT-PCR product was subcloned into pBluescript vector for sequencing and prope preparation. The product had a molecular weight of about 470 bp as estimated by polyacrylamide gel electrophoresis.--

Please replace the paragraph at page 39, line 17, to page 40, line 4, with the following paragraph:

--At postoperative day 14, samples were harvested from the bridge site of both ears. Total RNA was isolated using Totally RNA(Ambion, Austin, Texas, USA) according to the manufacturer's instructions. The RT was followed by a PCR reaction conducted in a total volume of 50µl that contained 1.5mM MgCl2, 10mM of each dATP, dCTP, dGTP and dTTP; 0.4 Units of Taq DNA polymerase(GibcoBRL). The primer pair used, designed on the basis of the coding cDNAs for rabbit VEGFR-3(this article) was: for sense 5'-TATGGTACAAAGATGAGAGGC-3' (SEQ ID NO: 5), and for antisense 5'-ACAGGTATTCACATTGCTCCT-3' (SEQ ID NO: 6). The PCR with this pair of primer yielded 362bp reaction product, and was tested with cDNAs of various rabbit tissues(lung, liver, mesentery, lymph nodes) to test the specificity before proceeding to the quantitative RT-PCR. In order to quantify the VEGFR-3 mRNA product in both VEGF-C treated and control ears, we used the "competimer" quantitative PCR technique: VEGFR-3 cDNA and 18S cDNA were co-amplified at the same time for each sample. In the same mix with VEGFR-3 PCR we added a mix of 18S primer pair/18S 3'-end modified

primers(competimers) at a ratio of 1/9(Ambion, Austin, Texas), yielding a 488-bp product. After forty cycles of PCR with the above condition, PCR products were separated on agarose gel containing ethidium bromide and quantified by using integrated density analysis software(EagleSight Software 3.2, Staratagene, La Jolla, CA, USA). RT-PCR and relative quantification of PCR products were performed at least three times on samples from both treated and contralateral ears(n=5 in each group) .--

REMARKS

Applicants request the Examiner to enter the changes in the specification requested above. These changes are being made pursuant to the Notice to File Missing Parts for Nonprovisional Application, mailed November 9, 2001, containing a request for a sequence listing.

Applicants submit herewith Sequence Listing, pages 1-6, to include as a sequence listing as part of this Application.

Applicants have amended the Application to include the sequence identification number in the specification where reference is made to the sequence. No new matter has been added by virtue of the amendment made to the specification.

Further enclosed is a computer readable copy of the above-mentioned copy of the Sequence Listing.

Also enclosed is a Statement in Support of Filing and Submissions in Accordance with 37 CFR 1.821-1.825 (2 pages), which declares that the content of the paper and the computer readable copies of the Sequence Listing are the same.

In view of the foregoing amendments and remarks, the present application is respectfully considered in condition for allowance. An early reconsideration and notice of allowance are earnestly solicited.

Although it is not believed that any additional fee is required to consider this submission, the Commissioner is hereby authorized to charge our deposit account no. 04-1105 should any fee be deemed necessary.

Respectfully submitted,

Date: June 5, 2002

Kathryn A. Piffat, Ph.D.

Kathryn A. Piffat, Ph.D. (Reg. No. 34,901)
Dike, Bronstein, Roberts & Cushman
Intellectual Property Practice Group of
EDWARDS & ANGELL, LLP
P.O. Box 9169
Boston, MA 02209
(617) 439-4444
(617) 439-4170 (fax)

APPENDIX I

REVISIONS OF THE SPECIFICATION PURSUANT TO REVISED RULE § 1.121

In the specification:

The paragraph at page 12, lines 19-21, should be replaced with the following paragraph:

--Figure 21 is a drawing showing a partial sequence of the rabbit VEGFR-3 cDNA sequence (SEQ ID NO: 7). Also shown, for comparison, are bovine (SEQ ID NO: 8), human (SEQ ID NO: 9) and mouse (SEQ ID NO: 10) sequences.--

The paragraph at page 12, lines 23-27, should be replaced with the following paragraph:

--Figure 22A is a drawing showing the amino acid sequence encoded by the rabbit nucleic acid sequence of figure 21 (SEQ ID NO: 11). Also shown for comparison are bovine (SEQ ID NO: 12), human (SEQ ID NO: 13), and mouse (SEQ ID NO: 14) sequences. Figures 22B-C show results of RT-PCT experiments. Results of those experiments are summarized in Figure 22D.--

The paragraph at page 38, lines 7-31, should be replaced with the following paragraph:

--Because the rabbit VEGFR-3 DNA sequence has not been disclosed, we sequenced part of the VEGFR-3 cDNA using degenerate oligonucleotides. Degenerate oligonucleotides were designed from conserved aa sequences NVSDSLEM (SEQ ID NO: 1) and WEFPRER (SEQ ID NO: 2), located 90 aa residues upstream or 40 aa residues downstream, respectively, of the trans-membrane domain of human and mouse VEGFR-3/Flt-4(Finnerty et al 1993, Galland 1993). The deduced oligonucleotide sequence were 5'-AACGTGAG(CT)GACTC(GC)(CT)T(AGCT)GA(AG)ATG-3' (SEQ ID NO: 3) and 5'-CC(GT)YTC (CT)C(GT) GGG(AG)AA(CT)TCCCA-3' (SEQ ID NO: 4), respectively.

Total RNA was extracted from kidney, ear, paraaortic lymph nodes, mesentery, and lung using TRIzol(Life Technologies, Inc., Grand Island, NY, USA) according to the standard acid-guanidium-phenol-choloroform method. Two microgram of total RNA were reverse transcribed using random hexamer and Moloney murine leukemia virus reverse transcriptase(MMLV-RT) (SuperscriptII™, GibcoBRL, Life Technologies, Inc., Grand Island, NY, USA) according to the manufacturer's instructions. Briefly, the RNA was reverse transcribed in 20μl of reaction mixture containing of 10mM of each dATP, dCTP, dGTP, and dTTP; 0.1M DTT; 200U MMLV-RT, 40U Ribonuclease inhibitor and buffer. One tenth volume of the reverse transcriptase(RT) product was subjected to polymerase chain reaction(PCR) in the presence of the above-mentioned pair of oligonucleotides and Taq DNA polymerase(GibcoBRL). PCR cycles were as follows: 94°C, 2min(once); 94°C, 15 sec; 50°C, 30sec; 72°C, 1 min(30 times); 72°C, 10 min(once). A single PCR product of approximately 470 base pairs was obtained from all the tissues The PCR product from the kidney sample was subcloned into the pBluescript vector(PCR-Script Amp Cloning Kit, Stratagene, La Jolla, CA, USA) for sequencing and probe preparation. Sequencing was performed utilizing simultaneous bidirectional-sequencing technique using Sequwncher(GeneCodes, Ann Arbor, MI)(MWG Biotech Inc., High Point, NC, USA).--

The paragraph at page 39, lines 8-14, should be replaced with the following paragraph:

--Figure 21 is explained in more detail as follows. Degenerate oligonucleotides designed from conserved amino acid sequences NVSDSLEM (SEQ ID NO: 1) and WEFPRER (SEQ ID NO: 2), located 90 amino acid residues upstream or 40 amino acids downstream of the transmembrane domain of human and mouse VEGFR-3 were obtained. Reverse transciption and PCR were conducted. The resulting RT-PCR product was subcloned into pBluescript vector for sequencing and prope preparation. The product had a molecular weight of about 470 bp as estimated by polyacrylamide gel electrophoresis.--

The paragraph at page 39, line 17, to page 40, line 4, should be replaced with the following paragraph:

--At postoperative day 14, samples were harvested from the bridge site of both ears. Total RNA was isolated using Totally RNA(Ambion, Austin, Texas, USA) according to the manufacturer's instructions. The RT was followed by a PCR reaction conducted in a total volume of 50µl that contained 1.5mM MgCl2, 10mM of each dATP, dCTP, dGTP and dTTP; 0.4 Units of Taq DNA polymerase(GibcoBRL). The primer pair used, designed on the basis of the coding cDNAs for rabbit VEGFR-3(this article) was: for sense 5'-TATGGTACAAAGATGAGAGGC-3' (SEQ ID NO: 5), and for antisense 5'-ACAGGTATTCACATTGCTCCT-3' (SEQ ID NO: 6). The PCR with this pair of primer yielded 362bp reaction product, and was tested with cDNAs of various rabbit tissues(lung, liver, mesentery, lymph nodes) to test the specificity before proceeding to the quantitative RT-PCR. In order to quantify the VEGFR-3 mRNA product in both VEGF-C treated and control ears, we used the "competimer" quantitative PCR technique: VEGFR-3 cDNA and 18S cDNA were co-amplified at the same time for each sample. In the same mix with VEGFR-3 PCR we added a mix of 18S primer pair/18S 3'-end modified primers(competimers) at a ratio of 1/9(Ambion, Austin, Texas), yielding a 488-bp product. After forty cycles of PCR with the above condition, PCR products were separated on agarose gel containing ethidium bromide and quantified by using integrated density analysis software(EagleSight Software 3.2, Staratagene, La Jolla, CA, USA). RT-PCR and relative quantification of PCR products were performed at least three times on samples from both treated and contralateral ears(n=5 in each group) .--